corrected for "trapped" plasma (4.5% by volume of cells) (*2*) and then converted into gravimetric percentage as follows: The specific gravity of plasma is 1.026 (average) and of whole blood containing 5,000,000 erythrocytes, 1.060 (*2*). The hematocrit of such blood is 43.2 by volume (*4*), corrected for "trapped" plasma to 41.3. If x = the specific gravity of cells, then $41.3x + (58.7)(1.026) = (100)(1.060)$. Solving, $x = 1.108$. If the volumetric hematocrit is h_1, and the gravimetric h_2, then

$$h_2 = 100\frac{1.108\,h_1}{1.108\,h_1 + 1.026(100 - h_1)}.$$

Solving,

$$h_2 = \frac{110.8\,h_1}{.082\,h_1 + 102.6}.$$

In calculating the specific heat of the cells, if x = the specific heat of the cells, K_3 = the determined specific heat of plasma, K_4 = the determined specific heat of the mixture of cells and plasma, and h_2 = the gravimetric hematocrit (corrected for "trapped" plasma) of this mixture in per cent, then $100\,K_4 = K_3(100 - h_2) + h_2x$.
Solving,

$$x = \frac{100\,(K_4 - K_3) + h_2K_3}{h_2}.$$

By this method the specific heat of cells, which may be redesignated K_5, was determined to be 0.77.

To test the accuracy of the method, the specific heats of several random mixtures of cells and plasma were calculated. If x = the specific heat of the mixture, and h_2 = the gravimetric hematocrit (corrected for "trapped" plasma) of that mixture, then $100\,x = (h_2 - 100)\,K_3 + h_2K_5$. The specific heat of this mixture was then measured directly and checked against the calculation. The results of four such calculations were as follows:

Sample No.	1	2	3	4
Hematocrit volumetric, uncorrected	32.4	42.4	41.6	30.0
Specific heat, calculated	0.89	0.87	0.87	0.89
Specific heat, actual	0.89	0.86	0.86	0.88

The small discrepancies between calculated and actual specific heats may be attributable to the error inherent in the method, and perhaps in part to minor variations in the hemoglobin content of the cells and in the percentage of solids, especially proteins, in the plasma. The correlation is, nevertheless, good, and the specific heat of any mixture of cells and plasma can be calculated from the hematocrit.

The low specific heat of cells in comparison with plasma is probably due to their greater concentration of solids, especially iron.

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Qualitative Reaction for 2,4-Dichlorophenoxyacetic Acid[1]

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There has arisen a need for a chemical method of identification of trace amounts of 2,4-D. Various bioassays employing plants have been proposed (*5*), as have methods of quantitative determination of the material, ranging from several gamma/ml to a gram and up (*1, 3, 4*). Bandurski (*1*) has employed a spectrophotometric method for the determination. However, quantitative methods of analysis are not always suitable for qualitative identification of a chemical.

To date, no satisfactory color derivative of 2,4-D has been developed that would serve as a quantitative means of identification (*5*). Various derivatives have been tried, including the nitro derivatives and their reduction analogs (*3*), but these have not proven satisfactory.

Certain characteristic salts (*3*) are formed by 2,4-D, the most distinctive of which is the uranyl salt. This salt is bi-axial in one plane with distinctive markings radiating pinnately from the axes. However, since the range of this method is 200–400 γ of material/ml, it is not capable of detecting really micro amounts.

Various means of identification have been tried in this laboratory with varying degrees of success. A halogen-substituted aromatic compound, if negatively substituted in the ortho or para position, will react with ammoniacal alcohol to form an amino derivative. The sodium-1-2-naphthoquinone-4-sulfonate will then detect the amino group (*2*). This reaction employing sodium-1-2-naphthoquinone-4-sulfonate was found to be reasonably effective in detecting 2,4-D, but lacked sensitivity and ease of application.

It was noted that when acid was warmed gently in concentrated sulfuric acid with 1,8-dihydroxy naphthalene 3,6-disulfonic acid (chromotropic acid), a characteristic wine-purple color resulted. As little as 0.05 γ of 2,4-D/ml could be detected by this test. As far as we are aware, this reaction has never been reported in the literature.

The procedure consists of introducing 2,4-D into a test tube and bringing it down to dryness. It has been found that benzene is the most suitable material for extraction of the 2,4-D for this reaction. Certain other solvents, notably alcohol, interfere slightly with the results. After bringing the material down to dryness, a few crystals of the chromotropic acid are introduced on the end of a micro spatula, 2 ml of concentrated sul-

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furic acid added, and the material heated in a glycerine bath at 150° C for 1½–2 min. If the material is overheated, a charring occurs which interferes with the results of the test. The color develops quite rapidly while heating, going through the stage of pink to purple to a very deep wine-purple color.

Attempts were made to employ a standard liquid reagent by dissolving the chromotropic acid in concentrated sulfuric acid, but this proved unsatisfactory since the reagent (chromotropic acid) decomposed upon standing with the sulfuric acid. An excess of chromotropic acid is needed for maximum color development.

Because of the extreme sensitivity of the reaction, it is of the utmost importance that the glassware used be absolutely free of the 2,4-D. The author has encountered difficulty in running blank reactions with test tubes that had been washed thoroughly with soap and hot water and put through rinses of clean water because of the trace of 2,4-D left in the reaction vessel.

TABLE 1

Acid	H_2SO_4	+ Chromotropic acid
Acetic	No color	Purple
Chloroacetic	" "	Weak pink
β-Brompropionic	Brown	Brown
Butyric	Pinkish-brown	Brown-reddish-pink cast
Maleic	No color	Brownish-yellow
Malic	" "	Yellow
Tartaric	Yellow	Yellow changing to bright green, finally to brown
Lactic	"	Yellow
Oxalic	No color	Light pink
Dinheptylacetic	Brown	Brown
Phenylacetic	Yellow	Yellow
2,4-Dichlorophenyl-acetic	No color	Pink
o-Chlorophenoxy-acetic	Pink	Wine-purple
p-Chlorophenoxy-acetic	No color	Reddish-pink
2,4-Dichlorophenoxy-acetic	" "	Wine-purple
2,4-Dibromophenoxy-acetic	" "	" "
Benzoic	" "	Brown
2,4-Dichlorobenzoic	Pink	Dark pink
4-Chloro-2-methyl-phenoxyacetic	No color	Brownish cast
2,4,5-Trichloro-phenoxyacetic	" "	Wine-purple
Pentachlorophenoxy-acetic	" "	" "
Indole acetic	Light pink	Light pink
Naphthalene acetic	No color	Pinkish-brown
Salicylic	Brown	Brown
Stearic acid	Yellow	Yellow
Phenylethyl acetate	"	"

This test is not entirely specific for the 2,4-D, as other organic acids will also give this color. However, it is sufficiently characteristic to make its use feasible in the identification of the 2,4-D, even when it is extracted from plant material.

Table 1 gives the characteristic reaction of a number of organic acids and one ester with both sulfuric acid and the sulfuric acid-chromotropic acid mixture.

It is interesting to note that, in the aromatic acids, only the halogen derivatives of these acids react to give a characteristic color. An example of this is the phenylacetic acid and the 2,4-dichlorophenylacetic acid. Some difference is noted also in the position of substitution. For example, the *o*-chlorophenoxyacetic acid gives a much deeper color with the reagent than the *p*-chlorophenoxyacetic acid.

While the mechanism of this reaction has not been determined—and so far we have used it only as a qualitative method—there does appear to be a possibility that the reaction could be put on a quantitative basis. We have found that, by employing varying quantities of 2,4-D, a more intense color results as the quantity of 2,4-D is increased. However, the technique is not sufficiently refined to enable one to determine the differences consistently between runs, and occasionally the relationship does not appear to be linear in the same run. This is probably due to the failure of the technique employed in carrying out the reaction.

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Proposed Method for Measuring the Movement of Soluble Fertilizer Salts in the Soil

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During the past 17 years the author has had an opportunity to use various types of lysimeters in studying the movement of soluble salts in the soil during the growing season. One of the uses made of the data collected from these lysimeters was to advise growers concerning the extent of leaching of plant nutrients from the soil. The installation of lysimeters and the collection and analyses of the leaching therefrom requires rather elaborate installation costs and detailed procedures.

In the past few years a simple system of measuring the movement of soluble salts in the soil has been under study and has been very valuable from the standpoint of recommending fertilization practices for different soil types. The system consists of sinking 10′ × 10′ frames of 12″ boards about 6″ in the soil. To the soil in these frames a definite amount of chloride ion is added by dissolving it in a given amount of water and sprinkling it uniformly over the soil. As many different conditions as desired can be established, and frames can be placed on as many different types of soil as conditions warrant.